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A Method to Predict the Recurrences of Acute Lymphoblastic Leukemia

[SPOSOB PROGNOZIRIVANIYA RETSIDIVOV OSTROGO LIMFOBLASTNOGO LEIKOZA]

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(54) A METHOD TO PREDICT THE RECURRENCES OF ACUTE LYMPHOBLASTIC LEUKEMIA

(57) **Abstract.** The invention concerns the field of medicine, especially hematology, and deals with the early pre-morphology immunodiagnosis of the recurrences of acute lymphoblastic leukemia (ALL). The purpose of the invention is to improve the accuracy of early detection of recurrences of the disease before manifestation of its clinical and morphological signs. The differentiation antigens characteristic of the patients' lymphoblasts in the acute phase of the disease are detected with a panel of monoclonal antibodies (MCA) in the reaction of indirect immunoflurescence; a panel of anti- T6, T9, T10, CALLA, Ia, IKO-11 MCAs has been developed in a microhole modification. The leucemic leukocytes are obtained from peripheral blood or bone marrow of the acute stage patients. The reaction is registered with a fluorescence microscope with additional phase contract observation of preparations. The antigens of lymphocytes obtained from the peripheral blood of patients during the periods of remission are examined in a similar way and with the same MCA panel. Examinations are repeated at regular intervals of 1 to 4 months over the whole period of remission. Appearance of lymphocytes positively reacting with even a single of the applied MCAs in concentrations exceeding their normal values is a precursor for the beginning of recurrence of the disease. The advantage of our proposed method is that based on our proposed MCA panel it is possible to recognize the leucemic cells in 96 % of patients and complete early pre-morphology detection of

leukemia recurrences for the maximal number of cases; such detection is based on the appearance of any of antigens characteristic of the acute-phase leucemic lymphoblasts on the "adult" lymphocytes.

The invention concerns the field of medicine, especially hematology, and deals with the early pre-morphology immunodiagnosis of the recurrences of acute lymphoblastic leukemia (ALL).

The purpose of the invention is to improve the accuracy of early detection of recurrences of the disease prior to manifestation of its clinical and morphological signs.

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The method is realized as follows:

The differentiation antigens characteristic of the patients' lymphoblasts in the acute phase of the disease are detected with a panel of monoclonal antibodies (MCA) in the reaction of indirect immunoflurescence. Examinations of the morphologically "adult" lymphocytes of peripheral blood are repeated at regular intervals of 1 to 4 months over the whole period of remission with the same MCA panel. Appearance of lymphocytes positively reacting with even a

single of the applied MCAs in concentrations exceeding their normal values (M + 3s) is a precursor for the beginning of recurrence of the disease.

Examinations of antigens of leucemic lymphoblasts are completed based on the reaction of indirect immunoflurescence in its microhole modification and using six MCA preparations included into the panel. The leucemic leukocytes are obtained after lysis of erythrocytes from 5 to 10 ml of heparinized peripheral blood or 1 ml of bone marrow taken from the acute-phase patients. The suspension is brought to the concentration of 5 x 10^6 cells per 1 ml of Hanks' (balanced salt) solution and attached to the glass surfaces of microholes with poly-L-lysin. In the beginning the cells are treated with the MCA preparations (20 ml each) at incubation within 30 minutes at 4° C and further, after double washing of microholes with the Hanks' solution, the cells receive the FITC-labeled anti- mouse g- globulin goat antibodies (20 ml each). Following to incubation within 30 minutes at 4° C the preparations are washed off twice with the Hanks' solution, and 5 ml of 50 % solution of glycerol is added into each microhole. The preparations are covered with cover grass and sealed off. The reaction is registered

with a fluorescence microscope followed by a checking phase contract observation of preparations. The differentiation antigens of lymphoblasts are detected based on the presence of cell surface luminescence. The antigens of lymphocytes obtained from the peripheral blood of patients during the periods of remission with a density-gradient centrifugation method are examined in a similar way and with the same MCA panel. Examinations are repeated at regular intervals of 1 to 4 months over the whole period of remission.

Case Study 1. Male patient "B", age - 15 years. Diagnosis: Acute lymphoblastic leukemia. The blast cells extracted from 5 ml of heparinized blood during the acute stage of the disease and examined using the our developed MCA panel, contained the Ia, IKO-11, CALLA, T10 and T9 antigens (the number of antigen-positive cells was equal to 91%, 55 %, 70 %, 90 % and 50 %, respectively). The T6 antigen on the patient's lymphoblasts was not found. As of the date of confirmed complete clinical and hematological remission 2 months after the number of morphologically "adult" blood lymphocytes, which contained the Ia, IKO-11, CALLA, T6, T9 and T10 antigens complied with the reference standard. However, 1 month after, still within the period of complete

clinical and hematological remission (at 3 % concentration of lymphoblasts in the bone marrow punctate) the repeated examination of lymphocytes with the same MCA panel have revealed increase in the number of cells with IKO-11 (68 %) and T10 (18 %) antigens at normal concentrations of lymphocytes, which contained Ia, CALLA, T6, and T9 antigens. Recurrence of the disease was found 1.5 months after at morphological examination of bone marrow punctate (concentration of lymphoblasts in the punctate was equal to 12.5 % and the disease continuously progressed after).

Case Study 2. Male patient "L", age - 18 years. Diagnosis: Acute lymphoblastic leukemia. The blast cells extracted from 5 ml of heparinized blood during the acute stage of the disease and examined using the our developed MCA panel, contained the Ia and IKO-11 antigens (the number of antigen-positive cells was equal to 70 % and 85 %, respectively). The CALLA, T6, T9 and T10 antigens on the patient's lymphoblasts was not found. One month after, within the period of clinically and morphologically confirmed complete clinical and hematological remission (at 4 % concentration of lymphoblasts in the bone marrow punctate) the examination of lymphocytes with the same MCA panel in parallel with

normal concentrations of cells containing the Ia, CALLA, T6, and T9 antigens, have revealed increase in the population of

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IKO-11 antigen-containing lymphocytes (46 %). Recurrence of the disease was found 3 months after at clinical and morphological examinations (concentration of blast cells in the peripheral blood was equal to 80 %).

The above case studies demonstrate that based on application of the earlier known method prediction of the recurrence of the disease is not possible. Thus, our proposed method improves the accuracy of immunological prediction of the ALL recurrences prior to any manifestations of their clinical and morphological signs due to application of several MCAs to different differentiation antigens detected on the leucemic lymphoblasts.

The method is applicable to all the ALL patients: with our developed MCA panel the leucemic cells are recognized in the great majority of cases (in 96 % of patients).

The Claim

A method to predict the recurrences of acute lymphoblastic leukemia through detection of differentiation antigens with the monoclonal antibodies on the leucemic lymphoblasts during the acute period of the disease and on lymphocytes at remissions in concentrations exceeding their normal values; such a prediction of disease recurrences is distinctive in that, for the purpose to improve the prediction accuracy, a set of specific monoclonal antibodies is applied for detection of antigens; detection of a single or several antigens points to recurrence of the disease in the near future.